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Diagnostic Work-Up and Risk Stratification in X-Linked Dilated Cardiomyopathies Caused by Dystrophin Defects

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Objectives We sought to describe the diagnostic work-up, phenotype, and long-term evolution of dilated cardiomyopathy (DCM) associated with *Dystrophin (DYS)* defects.

Background X-linked DCM associated with *DYS* defects can be clinically indistinguishable from other types of DCM.

Methods The series comprises 436 consecutive male patients diagnosed with DCM. Patients underwent endomyocardial biopsy (EMB). Genetic testing employed multiplex polymerase chain reaction and multiple ligation dependent probe assay for deletions and direct sequencing of the 79 exons and flanking regions of the gene for point mutations or small rearrangements.

Results We identified *DYS* defects in 34 of 436 patients (7.8%) (onset age 34 ± 11 years, age range 17 to 54 years); 30 had proven X-linked inheritance. The 2 phenotypes included DCM with mild skeletal myopathy and/or increased serum creatine phosphokinase (n = 28) or DCM only (n = 6). The EMB showed defective dystrophin immunostain. The *DYS* defects consisted of 21 in-frame deletions and 11 out-of-frame deletions as well as 1 stop and 1 splice-site mutation. During a median follow-up of 60 months (interquartile range: 11.25 to 101.34 months) we observed 17 events, all related to heart failure (HF) (median event-free survival: 83.5 months). Eight patients (23%) underwent transplantation, and 9 (26%) died of HF while waiting for transplantation. Eight patients received an implantable cardioverter-defibrillator, although none had device intervention during a median follow-up of 14 months (interquartile range: 5 to 25 months). No patient died suddenly, suffered syncope, or developed life-threatening ventricular arrhythmias.

Conclusions *DYS*-related DCM should be suspected in male patients with increased serum creatine phosphokinase (82%) and X-linked inheritance. The disease shows a high risk of end-stage HF but a lower risk of life-threatening arrhythmias. (J Am Coll Cardiol 2011;58:925–34) © 2011 by the American College of Cardiology Foundation

Dystrophin (*DYS*) (MIM*300377, Xp21.2) defects cause Duchenne Muscle Dystrophy (DMD), a severe myopathy affecting children and adolescents; Becker Muscle Dystrophy (BMD), a milder, delayed-onset myopathy with or without cardiac involvement; and an X-linked dilated cardiomyopathy (DCM) (MIM#302045) (1–12). *DYS* defects presenting with isolated or predominant cardiac involvement might be clinically indistinguishable from other types of idiopathic DCM, especially when the myopathy is subclinical, serum creatine phosphokinase (sCPK) is normal or mildly elevated, or the family is small and masks an X-linked recessive inheritance. *DYS*-related etiology can be suspected in male patients with DCM, increased sCPK, and absence of male-to-male transmission of the disease in the family. Genetic testing and endomyocardial biopsy (EMB) play a diagnostic role.

Most *DYS* defects causing DCM are deletions and cluster in 2 different regions of the gene: the 5' including muscle promoter-exon 1 and hinge region (3,5–7,9) and mid-rod domain exons (8,13,14). Prior comprehensive mutation

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Abbreviations and Acronyms

BMD = Becker Muscle Dystrophy
CHF = congestive heart failure
CI = confidence interval
DCM = dilated cardiomyopathy
DMD = Duchenne Muscle Dystrophy
DNA = deoxyribonucleic acid
DYS = Dystrophin gene
EMB = endomyocardial biopsy
HF = heart failure
HTx = heart transplantation
ICD = implantable cardioverter-defibrillator
IQR = interquartile range
LVEF = left ventricular ejection fraction
MLPA = Multiple Ligation-Dependent Probe Amplification
NSVT = nonsustained ventricular tachycardia
PCR = polymerase chain reaction
sCPK = serum creatine phosphokinase

scanning of the exons and splice junctions of *DYS* in patients with sporadic DCM without clinical evidence of skeletal myopathy identified putative point mutations in 3 of 22 male patients (13.6%) (15). In X-linked DCM, the pattern of expression of *DYS* mutations in cardiac muscle differs from that observed in skeletal muscle, suggesting relevant differences of gene processing as well as differences in the functional domains more relevant for one tissue versus the other (review in Cohen and Muntoni [16]).

Most data on the natural history of X-linked DCM associated with *DYS* defects come from the myology setting, namely from patients who presented to clinical attention because of myopathy. Left ventricular (LV) dilation and reduced left ventricular ejection fraction (LVEF) occur in less than one-third of the patients diagnosed with BMD (14,17), and data on the arrhythmogenic risk of X-linked DCM are limited. The present study sought to describe the prevalence, diagnostic work-up, phenotype, and long-term evolution of X-linked DCM associated with *DYS* defects.

Methods

From January 1995 to December 2009, we screened *DYS* in 436 unrelated male probands diagnosed with DCM. The inclusion criteria were male sex and DCM diagnosis according to World Health Organization criteria (18). Because the female carriers of *DYS* defects might develop late-onset DCM, we only excluded probands from families with male-to-male transmission. The diagnostic work-up in probands consisted of clinical examination, biochemical investigation (total sCPK, sCPK cardiac isoform [myocardial band], lactacidemia, and lactic dehydrogenase), electrocardiography, chest x-ray, 2-dimensional and Doppler echocardiography, 24-h Holter recording, right heart catheterization, coronary angiography (after the age of 30 years), right ventricular EMB, and search for viral genomes (Methods in Online Appendix). Neuromuscular assessment focused on detailed clinical history, physical examination, and neuromyological examination. Skeletal myopathy was defined by increase in serum CPK and/or muscle weakness. When possible, we traced the clinical and pathological records and autopsy samples of deceased relatives. Informed and consenting family members underwent clinical screening, genetic counseling, and testing (19). The carrier status of the parents and maternal relatives was assessed with microsatellite analysis (12). The disease was defined as "proven familial," when clinical screening and genetic testing documented a mutated mother, a mutated maternal aunt or uncle, maternal female carriers, and affected male cousins; the family history was specified as "unknown" when mothers or maternal relatives could not undergo clinical and genetic screening. Additional data on families are available in the Online Appendix. The clinical and research work-up is part of a broader program dedicated to familial cardiomyopathies and has been approved by the Institutional Ethical Committee.

Genetic testing. Genetic testing was performed in deoxyribonucleic acid (DNA) isolated from peripheral blood leukocytes. Until 2006, analysis of the *DYS* gene was performed with multiplex and single polymerase chain reaction (PCR) assays (including the muscle promoter and exons 2 to 8, 12, 13, 16, 17, 19, 29, 32, 34, 41 to 55, 58, 60, 61, 64, 66, 68 to 70, and 76), as previously described (19) (Online Fig. 1a). Since 2007, we have introduced Multiple Ligation-Dependent Probe Amplification (MLPA) analysis that allows the simultaneous hybridization and ligation of several probes in a single reaction tube, followed by PCR amplification and analysis by capillary electrophoresis. The MLPA analysis was carried out according to the recommendations of the manufacturer (MRC Holland, Amsterdam, the Netherlands) (20). The MLPA data were analyzed with GeneScan 3.7 software (Online Fig. 1b). All deletions identified in multiplex PCR assays and MLPA were confirmed with single exon PCR. The 79 coding and flanking regions of the gene were analyzed by direct, bidirectional sequencing in the DNA of patients who tested negative with MLPA and showed abnormal *DYS* immunostain of the myocytes in the EMB (Online Fig. 1c). Genomic sequences were derived from the Leiden Muscular Dystrophy Database. The MLPA analysis was performed in 118 control DNA samples from non-affected male subjects (age 36 ± 14 years, age range 18 to 55 years).

EMB. Right ventricular EMB was performed in all patients as per standard protocol in a referral center for heart transplantation. The EMB samples were processed for light microscopy and ultrastructural study (12). The immunohistochemical study was performed with antidystrophin monoclonal antibody at a dilution of 1:50, pH 9.9 (Monosan, Sanbio B.V., Uden, the Netherlands, Clone Dy4/6D3, Rod domain) according to the standard avidin–biotin–peroxidase complex method. The immunohistochemical study on paraffin sections was informative, independent of the type of mutation, and substituted, in the first-level routine diagnostic activity, for the more complex immunohistochemical stains with anti–C-terminus, anti–N-terminus, and anti–Rod-domain antibodies on frozen sec-

tions (12). We used, as control subjects, 22 EMBs from donor hearts obtained before transplantation (HTx) and EMBs from DCM patients with a negative genetic test for *DYS*.

Statistical analysis. Descriptive statistics were computed as mean ± SD or median (25th to 75th percentiles) for continuous variables and as absolute frequency and percentage for categorical variables. For the purpose of analysis, age at diagnosis was dichotomized at its median value. Event rates (death plus HTx)/100 person-year and 95% confidence intervals (CIs) were calculated. Kaplan-Meier method was used to compute cumulative event-free survival. Event-free survival curves of a series of potential predictors were compared by means of the log-rank test. No multivariable analysis could be performed, given the limited number of events. To better characterize the occurrence over time of cardiac death or heart transplant in these patients, we further described these events with the framework of competing risks; for this purpose we computed and plotted the cumulative incidence (at the end of the follow-up) for each of these competing events (21). Stata software (version 10.1, StataCorp, College Station, Texas) was used for computation. A 2-sided p value < 0.05 was considered statistically significant.

Results

Study population and baseline characteristics. The inheritance was X-linked recessive in 30 of the 34 families (88%). Data from family and evaluation of living mothers were not available in 4 cases (12%). The clinical phenotype was DCM with skeletal muscle involvement (either with elevated sCPK and muscle weakness [n = 16] or increased sCPK only [n = 12]) in 28 patients and solely DCM in 6 patients.

The mean age at clinical diagnosis of DCM was 34 ± 11 years, whereas the mean age at the diagnosis of *DYS*-related DCM was 35 ± 12 years (range 17 to 54 years). Eight of the 34 patients (23%) had a documented episode of the flu, shortly before the onset of DCM; these 8 patients were clinically diagnosed with myocarditis initially, but their EMB excluded inflammatory infiltrates. Quantitative PCR excluded the presence of viral genomes in the EMB samples and peripheral blood.

Electrocardiographic study showed nonspecific features (Table 1). Four (12%) patients had documented episodes of nonsustained ventricular tachycardia (NSVT) on Holter monitoring (Table 1). None had syncope. Baseline echocardiographic study showed mean LV end-diastolic diameter of 70 ± 9 mm and LV end-diastolic volume of 279 ± 83 ml (Table 1). The LVEF was 30 ± 11%. Right ventricular dimensions (mean right ventricular diameter 25 ± 2 mm) and function (mean tricuspid annular plane systolic excursion 19 ± 2) were preserved. The treatment included beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin II antagonists, furosemide, antialdosteronic agents, digoxin, amiodarone, anticoagulants or antiplatelets, and nitrates (Table 2).

Genetic findings. We identified 32 deletions and 2 point mutations (Table 1). Of the 32 deletion defects, 11 were out-of-frame, whereas 21 were in-frame (Leiden Muscular Dystrophy pages—reading-frame checker version 1.9). Most deletions involved the mid-rod domain region; deletions exclusively affected regions spanning between exons 45 and 55 in 28 of the 30 cases. Of the 2 point mutations, one predicted a premature stop codon at p.Arg1763 (UMD-1710) and another one affected the splice site at −2 of intron 13 (Table 1). Healthy control subjects tested negative with MLPA; in particular, we did not find exon deletions in control subjects.

EMBs. The control EMBs from both normal hearts of donors before transplantation and DCM without *DYS* defects showed homogeneous and intense sarcolemmal immunostain (Figs. 1 to 3). The EMBs from patients with *DYS* defects showed variable decreases of intensity of the immunostain and multifocal loss of dystrophin expression at the myocyte membrane level involving entire or segments of the sarcolemma, and as such, the immunostaining defects were either diffuse or patchy. The EMBs from the 3 patients with single deletion of exon 48 showed defective dystrophin immunostaining, as did EMBs of other patients with different mutations (Fig. 4).

Outcome and prognostic stratification. Of the 34 affected patients, 17 developed a major event over a median follow-up time of 82 months (interquartile range [IQR]: 29 to 125 months): 8 patients underwent HTx (mean LVEF 16%, range 10% to 20%) 23 months (IQR: 6 to 72 months) after diagnosis, and 9 died of congestive heart failure (CHF) 42 months (IQR: 15 to 81 months) after diagnosis while awaiting HTx. The mean age at the event was 41 ± 13 years (range 17 to 63 years); patients who underwent HTx were younger (mean age at diagnosis 29.5 ± 12 years, range 17 to 52 years) than those who died (mean age 42 ± 10 years, range 19 to 55 years). Of the 8 HTx patients, 4 had pure DCM with no clinical signs of associated myopathy and normal sCPK values (66, 87, 76, and 176 mU/ml, respectively), whereas 4 had increased sCPK (mean sCPK 601 ± 253 mU/ml, range 342 to 908 mU/ml). One of the 8 patients died of acute rejection 1 year after HTx: he had refused immunosuppressant treatments, and clinical and biopsy controls. Other HTx patients are alive and well. Eight patients received an implantable cardioverter-defibrillator (ICD) (mean age 36 ± 6 years, range 31 to 46 years); 1 underwent HTx 4 months later. The clinical indications for ICD implantation were: severely decreased LVEF and a single run of NSVT during an acute episode of heart failure in 1 patient; decreased LVEF and 1 run of NSVT recorded at Holter monitoring in a second patient; and LVEF <30% in 6 patients. There were no appropriate interventions of the devices during a median follow-up of 14 months from implantation (IQR: 5 to 25 months).

Table 1 Baseline Demographic, Clinical, and Genetic Data

Case #	Age at 1st Diagnosis (yrs)	Family History	Flu-Like Episode	ECG	LVEF (%)	LVEDD (mm)	NYHA Functional Class	NSVT	ICD	sCPK mU/ml (Normal Values ≤200)	Events	*Dystrophin* Deleted Exons and Point Mutations	*Dystrophin* In- and Out-of-Frame Del; Other Mutations
1	17	1	0	ALVR	23	68	II	0	0	880	0	3–16	In-frame
2	27	NA	0	ALVR	20	69	III	0	1	850	0	45–49	In-frame
3	37	1	0	ALVR	28	76	II	0	0	813	CHF death	2–7	In-frame
4	43	2	0	Infer-Q-wave	30	65	II	0	0	850	0	45–47	In-frame
5	45	2	0	ALVR	40	68	II	0	0	850	0	45–47	In-frame
6	25	1	1	LBBB	15	67	III	0	0	66	HTx	48	In-frame
7	39	2	0	ALVR	40	64	II	0	0	657	CHF death	48	In-frame
8	27	2	0	ALVR	40	63	II	0	0	545	0	45, 47	Out-of-frame
9	17	1	0	ALVR	15	80	III	0	0	908	HTx	6, 8, 12, 13, 16, 17, 19, 44	Out-of-frame
10	30	2	0	PM	45	65	II	0	0	243	0	45, 47–48	Out-of-frame
11	29	2	0	ALVR	32	61	III	0	0	421	CHF death	45, 47	Out-of-frame
12	48	2	0	LBBB	23	76	III	1	0	672	CHF death	48–54	Out-of-frame
13	38	1	0	LBBB	30	75	III	1	1	367	CHF death	48	In-frame
14	23	1	1	LBBB + AVB,1st	20	83	IV	0	0	342	HTx	45–48	In-frame
15	24	2	0	ALVR	45	74	II	0	0	675	0	45–47	In-frame
16	54	2	0	LBBB	20	78	III	0	0	456	CHF death	48–55	In-frame
17	32	1	1	LBBB	24	77	III	0	0	87	HTx	3, 4, 6	Out-of-frame
18	52	2	0	ALVR	28	75	III	0	0	76	HTx	45–48	In-frame
19	49	NA	0	LBBB+ AVB,1st	23	80	III	0	0	121	CHF death	45–52	Out-of-frame
20	26	1	1	Infer-Q-wave	39	64	I	1	1	2,772	0	48–49	In-frame
21	40	1	0	ALVR	50	62	I	0	0	601	0	45–54	Out-of-frame
22	21	1	0	ALVR	50	52	I	0	0	765	0	p.(Arg1763X)*	Nonsense
23	24	NA	0	ALVR	30	59	II	0	0	890	0	45–46	In-frame
24	29	1	0	LBBB	20	82	III	0	1	456	HTx	48–51	In-frame
25	17	1	1	Infer-Q-wave	22	73	IV	0	0	697	HTx	45–48	In-frame
26	19	1	1	LBBB	20	83	IV	0	1	112	CHF death	48–49	In-frame
27	19	1	0	ALVR	50	52	I	0	0	256	0	45–48	In-frame
28	42	NA	1	LBBB	20	78	III	0	0	176	HTx	IVS13-2A>T†	Splice site†
29	44	1	0	Infer-Q-wave	35	57	II	1	0	955	0	45, 47–51	Out-of-frame
30	41	2	0	PM	25	71	II	0	0	501	CHF death	45, 47–48	Out-of-frame
31	35	1	0	ALVR	40	59	II	0	0	757	0	45–46	In-frame
32	44	1	0	LBBB	20	59	II	0	1	980	0	46–48	Out-of-frame
33	29	1	0	PM	30	78	II	0	1	3,370	0	45–55	In-frame
34	37	1	1	ALVR	15	82	III	0	1	620	0	45–47	In-frame

Family history: 1) = proven in mothers (n = 17), or 2) = maternal relatives (n = 13). *(UMD-1710); †at IVS13-2, the c.1603-2A>C is reported (UMD-1710) in Becker Muscle Dystrophy (BMD) associated with dilated cardiomyopathy (DCM) and cramps and weak dystrophin immunostain; predicted p (Val535_Gln568del, Val535_Ala604Del).

ALVR = abnormal left ventricular repolarization; AVB,1st = first-degree atrioventricular block; CHF = congestive heart failure; Del = deletion; ECG = electrocardiogram; HTx = heart transplantation; ICD = implantable cardioverter-defibrillator; LBBB = left bundle branch block; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; NA = nonavailable deoxyribonucleic acid samples from mothers and other maternal relatives; NSVT = nonsustained ventricular tachycardia on Holter monitoring; NYHA = New York Heart Association functional class; PM = pacemaker; sCPK = serum creatine phosphokinase.

Table 2 Baseline Characteristics of the 34 *DYS* Mutation Carriers According to Their Cardiologic Status, and Medical Treatment at Baseline Diagnosis and at End of Follow-Up

sCPK (mU/ml)	700 ± 670 (66–3,370)	
LVEF (%)	30 ± 11 (15–50)	
LVEDD (mm)	70 ± 9 (52–83)	
LVEDV (ml)	279 ± 83 (136–475)	
RV diameter (mm)	25 ± 2 (22–31)	
TAPSE (mm)	19 ± 2 (17–26)	
NYHA functional class		
I	4 (12%)	
II	14 (41%)	
III	13 (38%)	
IV	3 (9%)	
III–IV	16/34 (47%)	
Treatments	Baseline	End follow-up
Beta-blockers	9/34 (25%)	30/34 (88%)
ACE inhibitors	13/34 (38%)	30/34 (88%)
ATI antagonists	1/34 (3%)	7/34 (21%)
Furosemide	10/34 (29%)	27/34 (79%)
Digitalis	4/34 (12%)	15/34 (44%)
Antialdosteronic agents	7/34 (21%)	19/34 (56%)
Amiodarone	2/34 (6%)	5/34 (15%)
Anticoagulants/platelet antiaggregants	11/34 (32%)	29/34 (85%)
Nitrates	1/34 (3%)	3/34 (9%)

Values are mean ± SD (range), n (%), or n/N (%).

ACE = angiotensin-converting enzyme; ATII = angiotensin II; LVEDV = left ventricular end-diastolic volume; RV = right ventricular; TAPSE = tricuspidal annular plane systolic excursion; other abbreviations as in Table 1.

The event rate for combined death and HTx was 9.1/100 person-year (95% CI: 5.7 to 14.7), with a median event-free survival of 81 months, whereas the death rate was 4.8/100 person-year (95% CI: 2.51 to 9.3). The cumulative event-free survival was 75% (95% CI: 56% to 86%) at 2 years and 64% (95% CI: 45% to 78%) at 5 years. Figure 5A shows the Kaplan-Meier curve of the combined events (death or HTx). At univariable analysis (Table 3), a lower LVEF, larger LV end-diastolic diameter, higher New York Heart Association functional class, normal sCPK, and absence of myopathy were all associated with an increased risk of events. With the analysis for competing risks, the cumulative incidence of cardiac death (all end-stage heart failure [HF]) and HTx at the end of follow-up were 36% (95% CI: 18% to 54%) and 28% (95% CI: 13% to 45%), respectively; the risk was substantially constant up to 96 months (as shown by the constant slope of the cumulative incidence curve in Fig. 5B) and similar for both outcomes.

Additional data on families are available in the Online Appendix.

Discussion

X-linked DCM associated with *DYS* gene defects constitutes <10% of all DCM in a consecutive male series.

Diagnosis. The contributory clinical markers for specific diagnosis of *DYS*-related DCM were male sex, increased sCPK and absence of male-to-male transmission of the disease in the families. These markers recur in more than 80% of the patients. The age of onset does not help, because the age at diagnosis might go beyond 50 years (14,19). Another supporting clinical marker is the presence of deep Q waves in inferior leads that has been previously described in DMD and BMD (22). However, only 4 of our patients had these electrocardiographic changes. The EMB plays a fundamental role documenting the loss of dystrophin expression in the myocardium, and genetic testing concludes the diagnostic work-up. Routine cardiac magnetic resonance imaging would contribute to better characterization of the distribution of fibrosis in the LV inferolateral wall (23).

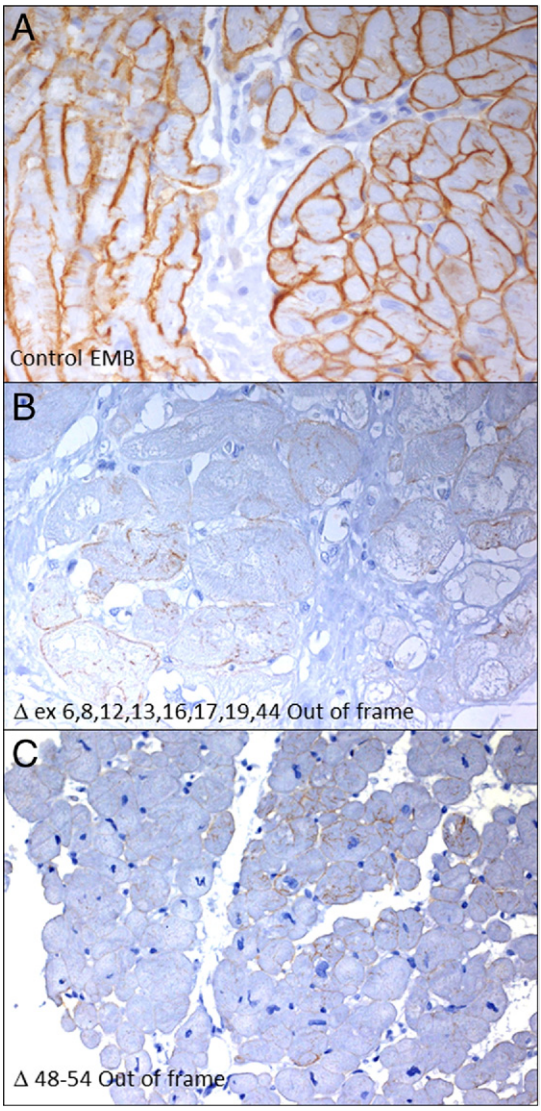

Figure 1 Out-of-Frame Deletions Versus Control: Defective Immunohistochemical Expression of Dystrophin

(A) Control endomyocardial biopsy (EMB) showing regular and intense immunostain of the sarcolemma; **(B)** EMB performed at the time of diagnosis in Patient #9 and **(C)** Patient #12 (Table 1), both carriers of different out-of-frame deletions, showing severe decrease of the dystrophin immunostain and cardiomyopathic changes (more prominent in the heart of Patient #9).

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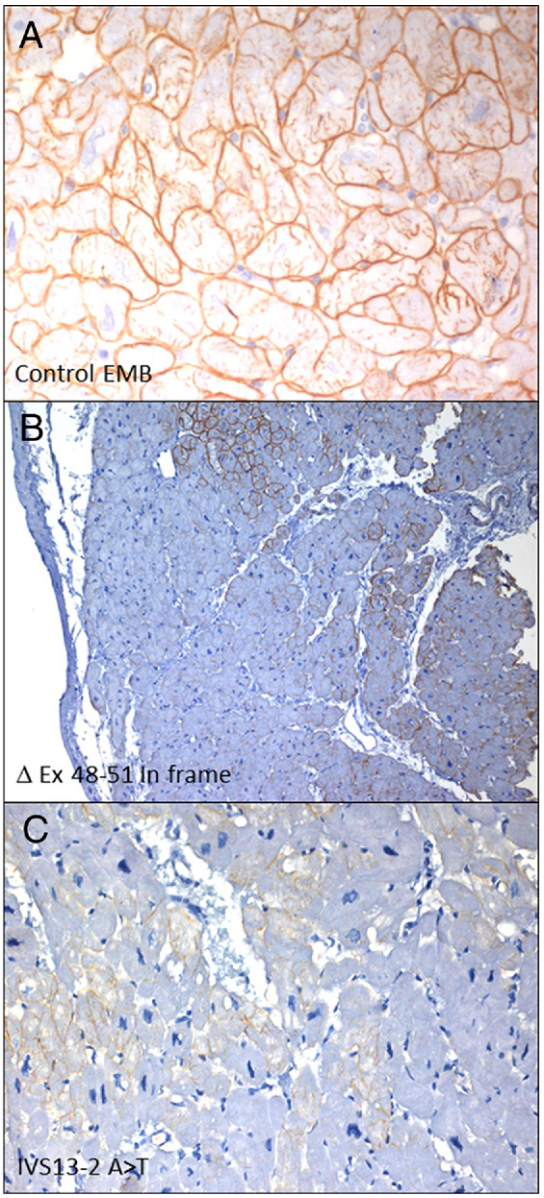

Figure 2 **In-Frame and Splice Site Mutations: Defective Immunohistochemical Expression of Dystrophin**

(A) Control dilated cardiomyopathy showing regular and intense immunostain of the sarcolemma; **(B)** endomyocardial biopsy (EMB), Patient #24 (Table 1), carrier of an in-frame deletion; and **(C)** Patient #28, carrier of a splice site mutation. The loss of protein expression associated with in-frame deletions was variable, with groups of myocytes maintaining intense immunostain and large areas of myocardium showing loss of protein expression. The dystrophin expression associated with the splice site mutation was characterized by low intensity and extensive loss of the immunostain.

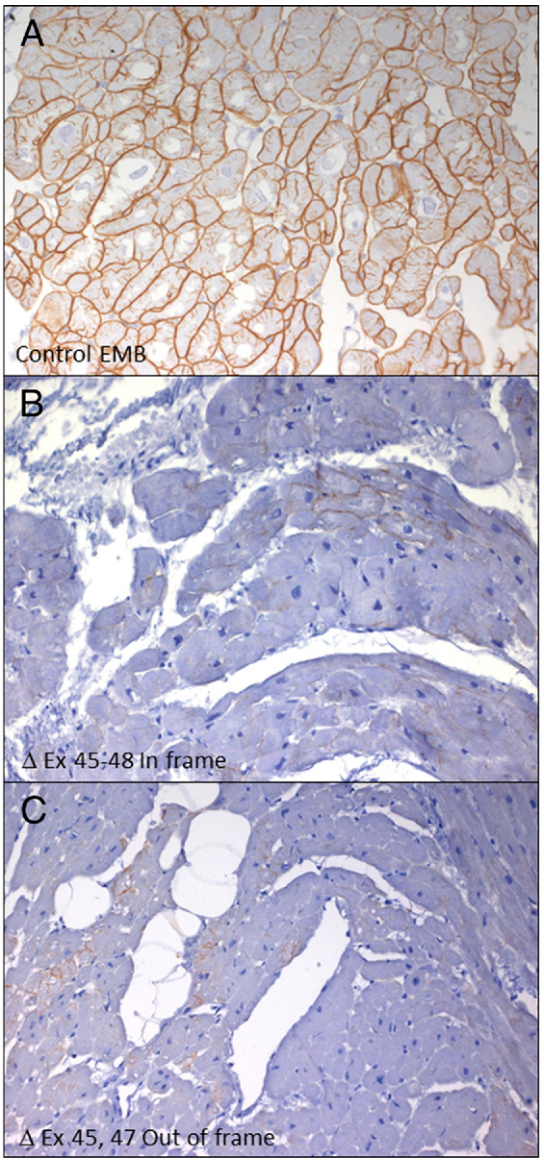

Figure 3 **In-Frame and Out-of-Frame Deletions: Defective Immunohistochemical Expression of Dystrophin**

(A) Control dilated cardiomyopathy showing regular and intense immunostain of the sarcolemma; **(B, C)** extensive loss of dystrophin in the endomyocardial biopsies (EMBs) from Patient #25, carrier of the in-frame deletion of exons 45 to 48 **(B)** and Patient #11, carrier of the out-of-frame deletion of exons 45 and 47 **(C)**. The comparative view of the dystrophin immunostain documents that the loss of protein expression can be severe and similar in the myocardium of patients with in-frame and out-of-frame deletions.

The history of a recent flu episode, observed in 8 of our patients, might mislead the clinical diagnosis. However, the possibility of a viral flu triggering the onset of a pre-existing asymptomatic disease or initiating clinical investigation of cardiovascular disease cannot be excluded. One of the 4 X-linked DCM cases reported by Neri et al. (24) had sudden cardiac failure after a flu-like illness. Although myocarditis and viral genome were absent in the EMB of our patients, we cannot exclude that the myocardium with *DYS* defects is more exposed to the damage induced by coxsackievirus proteases that can affect host cell proteins such as dystrophin (25).

Genetic tests should be performed—on the basis of present data that are the results of comprehensive immu-

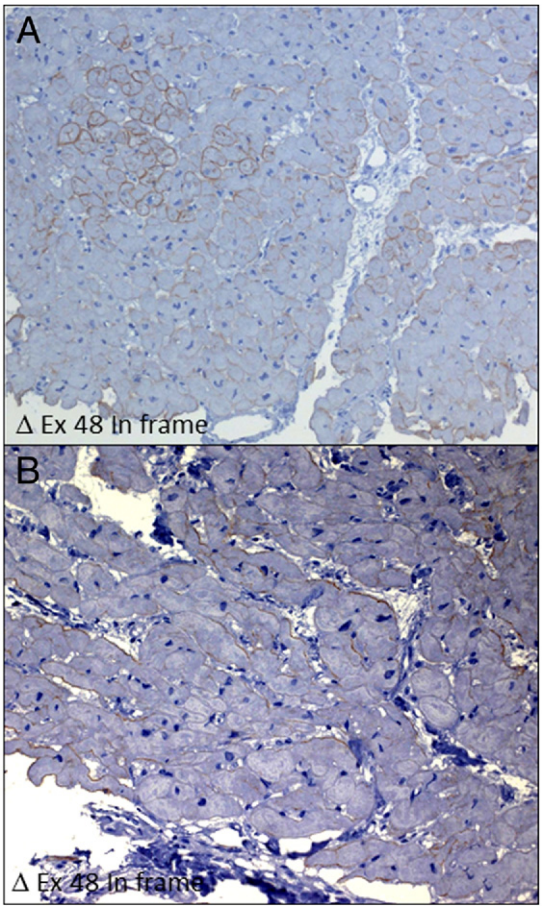

Figure 4 **In-Frame, Single Exon Deletion: Defective Immunohistochemical Expression of Dystrophin**

Endomyocardial biopsies of Patients #6 **(A)** and #13 **(B)**, carriers of single deletions of exon 48 (Table 1). The dystrophin immunostaining is defective in both cases and shows different severity of loss of the immunostain.

nohistochemical and genetic screening in male patients—in all patients with EMB showing defective *DYS* immunostaining. In fact, the results demonstrate that the loss of dystrophin expression in the affected myocardium is specific and sufficient to perform first-level *DYS* testing with MLPA. Direct sequencing of the coding and flanking regions of the gene should be performed when the EMB shows defective dystrophin immunostaining and MLPA is negative. The MLPA carries a high probability of detection of the genetic defect, because more than 90% of our patients with X-linked DCM showed deletions. The advantage of MLPA with respect to the previously employed multiplex PCR includes lower cost, greater time efficiency, and routine extension of the analysis to all 79 exons of the gene (including those whose deletions are extremely rare and are not usually assayed with multiplex PCR); it is also feasible in centers that do not perform EMBs (Fig. 3 in Online Appendix, Diagnostic work-up). For instance, MLPA did not identify deletions of exons that were not tested by our prior multiplex PCR, thus confirming that *DYS* deletions causing DCM cluster in the 5' region (3,5–7,9) and mid-rod domain exons (8,13,14) of the gene. In male patients who do not undergo EMB, we will maintain the strategy of *DYS* analysis with MLPA as first-level screening. In the case of negative results but evidence of X-linked inheritance in the family as well as either myopathy or increased sCPK in the proband or relatives, we will perform second-level genetic testing by direct sequencing of the coding and flanking regions of the gene. Next-generation sequencing techniques will likely provide the possibility of low-cost, extensive analysis of the gene.

Clinical evolution. In our series, there were no sudden cardiac deaths and syncope. Eight patients received the ICD for marked LV dilation and dysfunction. Only 2 of 8 patients had a single run of NSVT on Holter monitoring at baseline evaluation. None of the 8 patients had device intervention during a median follow-up of 14 months. Therefore, the arrhythmogenic risk seems to be much lower than that observed in other types of inherited DCM, such as cardiolaminopathies (26). By contrast, death for HF severely influenced outcome, because more than one-half of our patients either died of HF or needed transplantation. As shown by the cumulative incidence curve (Fig. 5A), the occurrence of both outcomes seemed fairly constant over the first 8 to 9 years of follow-up.

The prognostic factors for events were severe LV dilation and dysfunction and, paradoxically, the normal sCPK levels and absence of skeletal myopathy; this makes the diagnosis particularly difficult but offers useful prognostic information. The type of mutation does not correlate with the severity of the phenotype and disease evolution; the outcome of the 3 patients with in-frame deletions of single exon 48 was similar to that of patients with more complex deletions, both in-frame and out-of-frame. The effect of single deletion of exon 48 has been elegantly investigated in 5 male members of a single family by Morrone et al. (27), who documented the abnormal junction of exons 47 to 49 as well as the loss of exon 48 at the messenger ribonucleic acid level; of the 5 patients, 4 adults showed "sub-clinical DCM," whereas an 8-year-old boy had normal echocardiogram.

The long-term prognosis after transplantation seems to be excellent, confirming prior observations in solitary case reports or small series (28,29), with no impact of skeletal muscle involvement on the outcome. Therefore, patients with X-linked DCM associated with *DYS* defects confirm as good candidates for transplantation similar to other DCM patients.

Clinical advantages of a specific diagnosis. The correct diagnosis of X-linked DCM associated with *DYS* defects provides the basis for disease-specific considerations, such as the low arrhythmogenic risk and the high risk associated with end-stage HF. Treatments with statins and fibrates should be prevented before and after transplantation, because they might worsen the skeletal muscle status (30,31). Finally, genetic diagnosis allows extension of the testing in families, with identification of mutation carriers and healthy

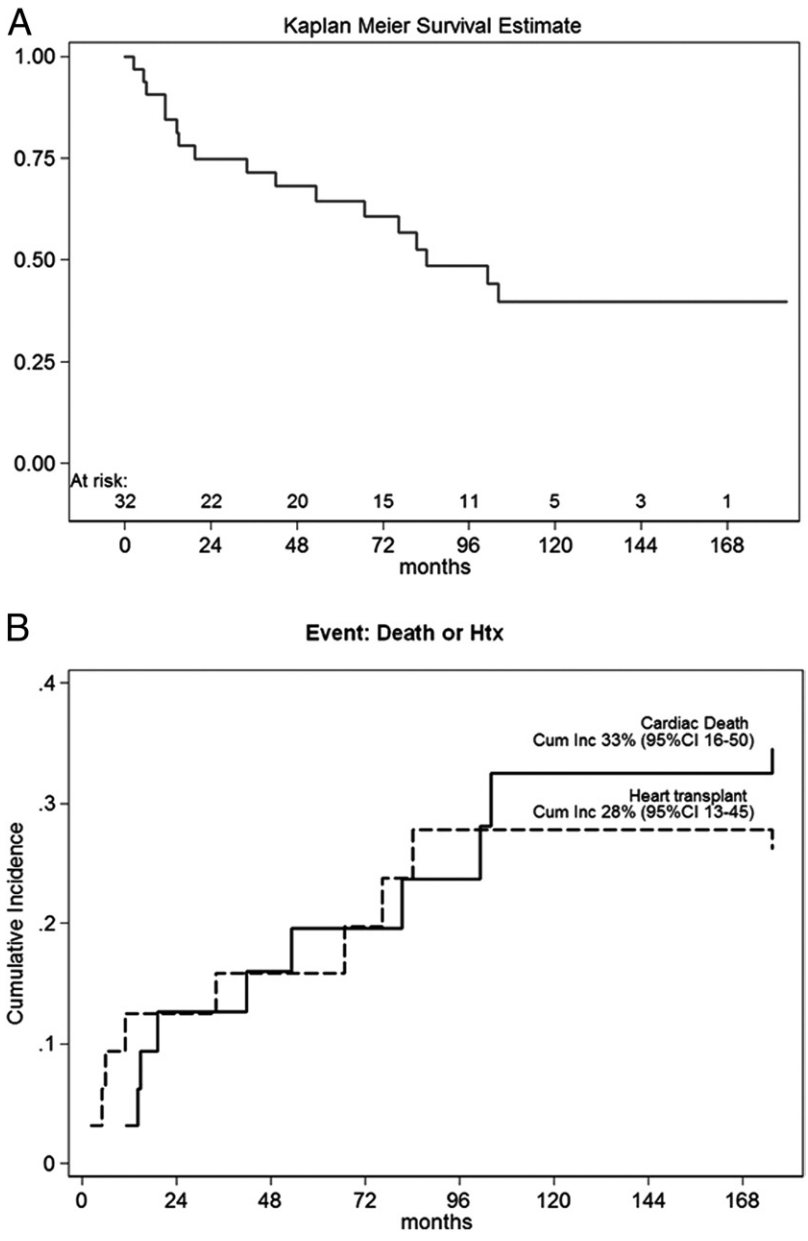

Figure 5 Kaplan-Meier Event-Free Survival and Cum Inc for Competing Events

(A) Kaplan-Meier event-free survival curve for the combined event of death and heart transplantation (HTx). The number of patients at risk is reported at the bottom of the figure immediately above the x-axis. **(B)** Cumulative incidence (Cum Inc) for the competing events of death and HTx. Cumulative incidence (Cum Inc) (95% confidence interval [CI]) at the end of the follow-up is reported for each outcome.

female and younger male relatives, as well as the possibility of pre-natal diagnosis.

Study limitations. The study is based on a relatively small number of patients; however, this in essence reflects the rarity of *DYS*-related DCM seen in routine cardiology practice; patients with DMD and BMD without DCM are usually diagnosed for the myopathic clinical pattern (DMD appearing earlier than the cardiomyopathy), even though DCM occurs in less than one-third of patients with BMD (14,17). Lastly, the present study was designed to validate the diagnostic work-up and to describe the long-term evolution of the disease in probands; the monitoring and follow-up of relatives are still ongoing.

Conclusions

Dilated cardiomyopathy associated with *DYS* defects should be suspected in the presence of male probands,

Table 3 Predictors of the Combined Endpoint of Death or Transplantation (n = 34)

	n	Events (% of Mutated)	Rate (95% CI) (Events/100 Person-Yr)	p Value (Log-Rank Test)
Age at first diagnosis				0.21
≤37 yrs	17	7 (41%)	7.02 (3.35–14.74)	
>37 yrs	17	10 (59%)	13.41(7.22–24.94)	
Flu-like episode				0.08
No	26	11 (42%)	7.63 (4.22–13.79)	
Yes	8	6 (75%)	19.94(8.96–44.39)	
sCPK				0.014
≤190 mU/ml	6	6 (100%)	26.15 (11.75–58.22)	
>190 mU/ml	28	11 (40%)	7.27 (4.03–13.14)	
LVEF				<0.001
<28%	18	14 (78%)	21.38 (12.66–36.10)	
≥28%	16	3 (19%)	2.76 (0.89–8.56)	
LVEDD				<0.001
≤70 mm	17	3 (18%)	2.70 (0.87–8.38)	
>70 mm	17	14 (82%)	22.16 (13.12–37.49)	
RV diameter				0.21
≤25	28	14 (50%)	8.78 (5.20–14.83)	
>25	6	3 (50%)	20.32 (6.55–63.01)	
TAPSE				0.39
≤19	22	13 (59%)	10.82 (6.28–18.64)	
>19	12	4 (33%)	7.40 (2.77–19.71)	
ICD insertion				0.53
No	26	14 (54%)	10.53 (6.23–17.78)	
Yes	8	3 (37%)	7.28 (2.34–22.57)	
NYHA functional class				<0.001
I	4	0	0	
II	14	3 (21%)	3.64 (1.17–10.29)	
III	13	11 (85%)	22.45 (12.43–40.53)	
IV	3	3 (100%)	25.23 (8.14–78.25)	
NSVT				0.52
No	30	15 (50%)	10.63 (6.41–17.64)	
Yes	4	2 (50%)	6.05(1.51–24.19)	
NYHA functional class III/IV				<0.001
No	18	3 (17%)	2.64 (0.85–8.21)	
Yes	16	14 (87%)	22.99 (13.61–38.82)	
Type of mutation				0.94
In-frame deletions	21	10 (48%)	10.75 (5.78–19.98)	
Out-of-frame deletions	11	6 (54%)	8.28 (3.72–18.43)	
Point mutations	2	1 (50%)	11.51 (1.60–81.70)	

CI = confidence interval; other abbreviations as in Tables 1 and 2.

X-linked recessive inheritance, and increased sCPK. The EMB documents the *DYS* defect, and genetic testing concludes the diagnostic work-up. *DYS*-related DCM is characterized by severe impairment of LV function, marked LV dilation, and low arrhythmogenic risk; the only factor that impacts survival seems to be end-stage HF.

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Key Words: congestive heart failure ■ dystrophin ■ heart transplantation ■ serum creatine phosphokinase ■ X-linked dilated cardiomyopathy.

APPENDIX

For supplementary text and figures, please see the online version of this article.